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(56) Documents Cited
EP 0358506 A2 WO 98/56897 A1
FASEB J. 1998, 12, pp.1331-1340 - Black et al.

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(54) Abstract Title
Bioabsorbable matrix for use as surgical/medical dressing

(57) An extracellular matrix is obtained by tissue culture of human umbilical vein endothelial cells on a matrix consisting of gelatin-fibronectin-heparan sulphate and, two days after confluence is reached, detaching the cells to leave behind their sub-endothelial or extracellular matrix. The latter is then used to culture and grow both dermal endothelial cells and dermal fibroblasts which again are eventually detached to leave behind a mixed extracellular matrix. The bioabsorbable matrix, after brief exposure to UV, may be sealed in an envelope for later use or may be enclosed in a gel-like substance which dissolves over time on contact with a patient's skin.

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TITLE

A SURGICAL-MEDICAL DRESSING FOR THE TREATMENT OF BODY BURNS AND FOR WOUND HEALING.

BACKGROUND

Wound coverage for patients with extensive burns has traditionally been provided by temporary grafts of cadaver skin. Since the development of techniques for tissue-culturing human epidermal keratinocytes, several dermal analogs to support keratinocyte growth have been the subject of research studies. Earlier studies had revealed that grafts composed of cultured cells and biopolymers dissolved rapidly when transplanted to athymic mice and humans. Also split thickness grafts, the gold standard for wound closure were limited by their unavailability in patients with total body burns.

In addition to body burns, diabetic ulcers, venous ulcers, pressure sores are but a few examples which affect large populations of patients. Available methods of treatment have proven inadequate, hence bioengineered skin substitutes provide the present focus for clinical investigation and evaluation. ORGANOGENESIS INC., of Canton, MA. employs a skin equivalent with an epidermal layer of cultured keratinocytes and a dermal analog of cultured neonatal fibroblasts in a collagen gel called Apligraf. ADVANCED TISSUE SCIENCES of La Jolla, CA.,are clinically testing Dermagraft which is a composite of allogenic neonatal fibroblasts grown in vitro on a bioabsorbable mesh. The above are but two examples.

Besides patients with body burns, characterised in ascending severity, as first, second, or third – degree, wound closure needs to be taken into account. Surgical stitching with sutures leaves the wound to heal itself with the passage of time. This results in the formation of granulation tissue and consequential scarring. No precursor of the complex cellular healing process is ever added.

PERTINENT SCIENTIFIC FACTS RELATING DIRECTLY TO THE INVENTION

In 1992, I published a scientific paper entitled ' The Seeding of Human aortic endothelial cells on the extracellular matrix of Human umbilical vein endothelial cells', in the 'International Journal of Experimental Pathology', Volume 73, pages 491-501. I was the sole author.
This work proved my assumption that since two independent groups of scientific researchers showed that the composite pattern of glycoproteins, including Type IV basement membrane collagen, thrombospondin, and fibronectin, secreted by cell cultures of Human adult vena cava and aortic cells, resembled the composite pattern of glycoproteins secreted by Human umbilical vein endothelial cells, all of these cells should be underlaid by a similar non-thrombogenic sub-endothelial or extracellular matrix. My paper demonstrated this.
Following the publication of my own studies, I came across a paper entitled ' The synthesis of extracellular matrix glycoproteins by cultured microvascular endothelial cells isolated from the dermis of Neonatal and Adult skin', published in the 'Journal of Cellular Physiology' 1985, Volume 123, pages 1-9, by R.H.Kramer, G.M.Fuh, K.G.Bensch, and M.A.Karasek. These researchers demonstrated that the human dermal skin endothelial cells secreted Type IV collagen, laminin, fibronectin, and thrombospondin from both the neonatal and adult skin endothelial cells.
The only difference between the human neonatal /adult skin endothelial cells and the human vena cava, aortic, and human umbilical vein endothelial cells is the additional secretion of laminin. The table below summarises this.

Human endothelial cells	Secretions
Aortic, Adult vena cava	Type IV collagen, thrombospondin, and fibronectin
Human umbilical vein	Type IV collagen, thrombospondin, and fibronectin
Neonatal/Adult skin	Type IV collagen, thrombospondin, fibronectin and laminin.

A few scientific words about laminin. Laminin has been shown to stimulate cell adhesion, growth, diffrentiation, and migration. This additional molecule found in Human skin will not prevent the adhesion of Human skin endothelial cells to the sub-endothelial or extracellular matrix of Human umbilical vein endothelial cells. This fact, allied with the reported, relative non-immunogenicity of the non-cellular components of the Human dermis, forms the scientific backgroundfor the creation of a medical-surgical wound dressing which may be used to treat patients with burns, and /or as a primary dressing for wound healing.

It should be noted that the human dermal fibroblasts in the papillary dermis of the skin secretes the extracellular matrix proteins of the human dermis and they are nourished by the skin capillaries/blood microvessels from which the human dermal endothelial cells are extracted for tissue-culture.

THE INVENTION

A bioabsorbable matrix consisting of Gelatin-Fibronectin-Heparan Sulphate will be used as a substrate for the tissue culture and growth to confluence of Human umbilical vein endothelial cells, obtained from a single umbilical cord.

Two days after confluence is reached , the cells will be detached in a manner which will leave behind intact, their sub-endothelial or extracellular matrix.

Human dermis, obtained from cadaver skin, cosmetic surgery, skin biopsies, etc., under clinical conditions, will be mechanically abraded to facilitate the tissue culture and growth of both dermal endothelial cells and dermal fibroblasts on the extracellular matrix of the Human umbilical vein endothelial cells. Since it is recognised in the scientific literature, that one cell type tends to overgrow the other, growth will be carefully monitored over a period of days. These cells will be similarly detached to leave a mixed extracellular matrix behind.

After a brief exposure to UV radiation, the bioabsorbable matrix will be sealed in an envelope containing a small amount of Dulbecco's phosphate buffered saline, calcium- and magnesium-free at a pH of 7.4 .

Antibiotics e.g. penicillin, streptomycin may be added to preclude bacterial growth on the bioabsorbable matrix during storage.

An additional protocol may be foreseen whereby the bioabsorbable matrix with the overlaid mixed extracellular matrix, is totally enclosed in a soft, pliable, gel-like substance which will dissolve over time on contact with the patient's skin. This would enable easy handling by physicians.

Before the dressing is used, it would have been, of course, subjected to the most stringent quality control and cytoxicity tests to absolutely determine its non-cytoxicity. The advantage of using a mixed non-cellular matrix is that, unlike other bio-engineered skin substitutes, incorporating viable human cells, the shelf life will be longer, if stored properly.

Summary

It is hoped that speedy wound closure would result from using this new surgical-wound dressing. Even if this dressing were to later dissolve on application to a patient's wound, it would position, directly, non-cellular components of the human dermis exactly where they would be needed to aid wound healing. These non-cellular components of the human dermis consist primarily of extracellular matrix proteins and collagen which have been shown to be relatively non-immunogenic. It is a known fact that when physicians use a split thickness skin graft, the so-called STSG, the degree of scarring and contracture of the grafted wound correlates inversely with the amount of dermis that is delivered in a STSG.

By using a mixed extracellular matrix, incorporated in this new dressing, composed of both endothelial cell and fibroblast non-cellular proteins, the healing growth of both epidermal and dermal cells will be actively encouraged.

It is envisaged that in the future, prior to any surgical procedure, which will require wound healing, a skin biopsy will be obtained. This will allow a standard bioabsorbable mesh, with an incorporated gelatin/heparin substrate, overlaid with the extracellular matrix of a Human umbilical vein, to become seeded with the patient's own dermal endothelial cells and fibroblasts, which will provide an autologous mixed extracellular matrix.

SCIENTIFIC REFERENCES IN ADDITION TO THOSE QUOTED IN THE WRITTEN TEXT

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CLAIMS-Number 1.

AN IMPROVEMENT IN THE DESIGN OF ARTERIAL GRAFTS

The identical principle outlined for the surgical-medical dressing suggests an improvement in the design of man made arterial grafts otherwise known as vascular prostheses. BOSTON SCIENTIFIC MEADOX grafts are supplied with a gelatin/heparin matrix. A human umbilical vein endothelial cell extracellular matrix may be overlaid, then a human aortic endothelial cell matrix may be overlaid as a second matrix. Most researchers in this field have not considered the superposition of TWO matrices. They have tried to precoat the graft with a constituent of the extracellular matrix e.g. fibronectin, or collagen Type IV, or laminin, or a whole blood preclot matrix- see Vohra R. et al in THE BRITISH JOURNAL OF SURGERY (1991)Volume 78(4). pages 417-420. As a result, attempts at the endothelial cell seeding of grafts have yielded poor results, due to poor cell retention on the graft.

CLAIMS -Number 2 REPAIR OF SCAR TISSUE.

As a result of this specification, it now becomes feasible to repair Scar Tissue. Scars may be surgically opened and the prevailing scar tissue excised. Alternatively, maggots may be used to clean out the newly created wound. Then a small strip of the bioabsorbable mesh incorporated with an autologous mixed extracellular matrix will be inserted. No sugical stitching will be employed. Instead, the two separate edges of the open wound will be drawn together by a tight adhesive bandage applied externally.

CLAIMS -Number 3. ATTACKING CANCEROUS TUMORS.

Dr. Kramer in his paper 'Metastatic tumor cells adhere preferentially to the extracellular matrix underlying vascular endothelial cells' leaves open the possibility that an attack on the extracellular matrix by specific targeting of molecules which are anti-, say either -Type IV collagen, and/or -thrombospondin, and/or -fibronectin, and/or -laminin, might provide a clinical means of shrinking cancerous tumors present in the human body.

CLAIMS-Number 4

TISSUE REPAIR

The analogy of helping the human body to react faster than normal, e.g. when a snake bite occurs, may also be drawn. The body starts to produce anti-bodies to counteract, but in an insufficient, ineffective quantity.
It becomes absolutely necessary to inject an anti-venom to save the patient's life.

External applied help in the form of a bioabsorbable mesh incorporating the non-cellular building blocks in the format of a mixed extracellular matrix should be helpful in alleviating clinical manifestations of non-healing wounds.

CLAIMS – Number 3

ALTERNATIVE DRESSING SKIN INTERFACE

Instead of enclosing the mixed extracellular matrix with a soft, pliable gel-like substance, an alternative might be envisaged. This consists of spraying the mixed extracellular matrix with an antibiotic, to wet it, and then applying 100% cornstarch powder, which will adhere. The powdered surface will serve to absorb oozing, wound fluid. Indeed, an antibiotic cornstarch powder could serve as a temporary first-aid treatment. This antibiotic powder might pre-empt a pure fibrin blood clot, leading to less scarring of the skin and less contracture.

CLAIMS – Number 6

A MODEL FOR THE STUDY OF IMMUNE REJECTION

In the event that the dermal endothelial cells, (and the dermal fibroblasts), do not adhere to the extracellular matrix of the human umbilical vein, this occurrence might serve as a simple model for the study of immune rejection.

Amended claims have been filed as follows

Additional Claim

Trypsinized human epidermal keratinocytes seeded onto HUVECs ECM adhered within twenty minutes and a confluent layer was achieved, Conditioned medium from human umbilical vein endothelial cells (HUVECs) was found to neutralize Dispase commonly used to detach epithelial sheets after the *in vitro* culture of cultured epithelial autografts (CEAs). These findings could lead to a shortened production time and better adhesion of CEAs used clinically for the treatment of burns patients. The same conditioned medium is specific for the growth of human dermal microvascular endothelial cells, human dermal fibroblasts and human epidermal keratinocytes.

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Claims searched: -

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Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.S):

Int Cl (Ed.7):

Other: Online: EPODOC, WPI, BIOSIS, MEDLINE, CAS-ONLINE, SCISEARCH, EMBASE

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	EP 0358506 A2	(MARROW-TECH INC.) see p.3 ll.7-30 and 45-47, p.7 ll.37-48 and p.11 ll.13-24	
X	WO 98/56897 A1	(FIDIA ADV. BIOPOLYMERS) see p.3 ll.9-29, p.6 l.23 - p.7 l.26, p.8 l.30 - p.9 l.12 and Examples 1-5	
X	FASEB J. 1998, 12, pp.1331-1340 "*In vitro* reconstruction .." - Black et al. - see Abstract, "Experimental Procedures" and "Results" on p.1333		

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.